577. *The Seed Fat of* Œnothera biennis *L.*

By J. P. Riley.

The structure—octadeca-6 : 9 : 12-trienoic acid *—proposed by Eibner, Widenmeyer, and Schild for the trienoic acid present in the seed oil of *Œnothera biennis* has been confirmed. The methyl ester of the acid has been isolated in a pure state by debromination of its hexabromide, and values are presented for its behaviour to alkali isomerisation under the conditions employed for the determination of triene acids of this type. The oleic and linoleic acids present in the fat have been shown to be the acids normally occurring in vegetable fats. The composition of the component mixed fatty acids has been shown by low-temperature crystallisation and spectrographic methods to be : palmitic 8·8, stearic 1·3, higher saturated acids 1·0, oleic 7·0, linoleic 71·7, and octadeca-6 : 9 : 12-trienoic acid 10·2% (wt.).

Œnothera biennis L., commonly known as the evening primrose, belongs to the order Myrtiflorae, family Onagraceae, and is a herbaceous perennial. It was introduced in 1614 into European gardens from Virginia, and has since become a wild plant. It is biennial and produces in the first year rosettes of leaves; in the autumn of the second year it forms tall stems, each bearing numerous yellow blooms, which, as the common name implies, open in the evening, being pollinated by moths. Below the flower there is a long seed pod, which is divided longitudinally into four compartments, each containing many tiny seeds.

The seed oil seems to have been first examined by Unger (*Apoth. Ztg.*, 1917, **32**, 351), who showed that the seeds contained 15% of oil extractable with light petroleum. Heiduschka and Luft (*Arch. Pharm.*, 1919, **257**, 33) extracted 14% of oil with ether, and were able to show that, besides normal oleic and linoleic acids, there was present in the mixed fatty acids a linolenic acid, yielding on bromination a hexabromide, m. p. 196° (decomp.), which they called γ-linolenic acid. Eibner, Widenmeyer, and Schild (*Chem. Umschau*, 1927, **34**, 312), on bromination of the mixed fatty acids in ether, obtained a hexabromide, m. p. 203°. On debromination, and ozonolysis of the resultant γ-linolenic acid, there were obtained, after hydrolysis of the ozonide, hexaldehyde and adipic acid, and they therefore concluded that γ-linolenic acid is octadeca-6 : 9 : 12-trienoic * acid.

Since this structure, suggested over twenty years ago, has remained unconfirmed, and since this acid has not been reported in any other natural fat, it seemed desirable to repeat the earlier observations with the aid of techniques more recently developed, and the present investigation was therefore directed towards this end and to the accurate analysis of the mixed fatty acids of the oil. For this purpose, plants growing wild on the sandhills near Southport, Lancashire, were

---

* In this paper the acids are numbered on the Geneva system, the number 1 being assigned to the carboxyl carbon atom (cf. this vol., pp. S 91, 1541).

collected and dried before the seeds were separated. In Table I are shown the characteristics of the oil extracted, together with those reported by other workers.

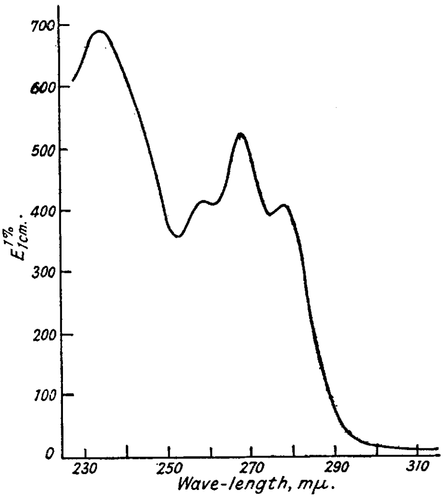
TABLE I.

Characteristics of Œnothera biennis *oil.*

	I.	II.	III.	IV.
Oil (on dried seed), %	11·1	*ca.* 14	*ca.* 21	9·1
Saponification equivalent	287·8	287·3	304·8	—
Iodine value (I.V.)	154·8	148·9	152·1	—
Free fatty acid (as oleic), %	1·1	0·0	—	—
Unsaponifiable matter, %	3·5	2·27	2·4	—
Refractive index, *n*	1·4782/25° 1·4740/40°	1·4722/40°	1·4782/20°	—

I, Present work.
II, Heiduschka and Luft (*loc. cit.*).
III, Ueno and Kimura (*J. Soc. Chem. Ind. Japan*, 1943, **46**, 486).
IV, Litvinchuk (*Tekh Kul'tari*, 1939, **19**, 75).

The mixed acids of the oil have been examined by low-temperature crystallisation from solvents, coupled with the determination of the component acids of the fractions by alkali isomerisation, by slight modification of the methods used by Gunstone and Hilditch (*J. Soc. Chem. Ind.*, 1946, **65**, 8) for the analysis of oils containing linoleic and linolenic acids. The most soluble crystallisation fraction, which contains the majority of the trienoic acid present in the oil, has been ozonised, and adipic acid detected among the hydrolysis products, confirming the deductions of Eibner *et al.* (*loc. cit.*) that the acid is octadeca-6 : 9 : 12-trienoic acid. For the purposes of the spectrographic analysis of the oil, it was necessary to obtain a pure specimen of the acid, in order to determine its behaviour to alkali isomerisation. This has been accomplished by the bromination of the concentrate of the acid, and debromination of the purified hexabromide, m. p. 196°, by means of zinc and pyridine. The methyl ester of the product was fractionally distilled, and on alkali isomerisation and spectrographic examination by the method of Hilditch, Morton, and Riley (*Analyst*, 1945, **70**, 67) the results (expressed as acids) shown in the figure and in Table II were obtained. In Table II are also recorded for comparison the values obtained under the same conditions by Hilditch *et al.* (*loc. cit.*) for normal linolenic acid (octadeca-9 : 12 : 15-trienoic acid).



*Ultra-violet absorption of methyl octadeca-*6 : 9 : 12-*trienoate after alkali isomerisation at* 170° *for* 15 *minutes.*

From the above table it will be seen that there is good qualitative agreement between the results for the two acids. The slight divergences may be explained by difference in either the positions of the double bonds or the *cis-trans*-configurations of the acids. In the calculations of the analysis of the *Œnothera* oil, the values obtained for the 6 : 9 : 12-trienoic acid have been employed.

Oxidation of a concentrate of the oleic and linoleic acids, obtained by low-temperature crystallisation, yielded the normal di- and tetra-hydroxystearic acids formed by the oleic and linoleic acids (octadec-9-enoic and octadeca-9 : 12-dienoic) found in vegetable fats. Further proof of this structure was afforded by the fact that acetone–permanganate oxidation of the concentrate yielded azelaic acid as the sole dibasic acid.

TABLE II.

Behaviour of octadecatrienoic acids on alkali isomerisation.

	6 : 9 : 12-Trienoic acid.		9 : 12 : 15-Trienoic acid.	
$E^{1\%}_{1 cm.}$ at	234 mμ.	268 mμ.	234 mμ.	268 mμ.
Isomerised at 170° for 15 mins.	690	522	622	532
Isomerised at 180° for 60 mins.	603	—	569	482

Palmitic acid has been identified as being the principal saturated acid present in the oil, and is associated with small amounts of stearic acid and of a mixture of higher saturated acids which has not been resolved.

In Table III is shown the composition of the mixed acids of the oil determined by the procedure outlined above, together with the values recorded by Heiduschka and Luft (*loc. cit.*) and by Eibner and Schild (*Chem. Umschau*, 1927, **34**, 339).

The agreement between the three sets of results is as good as can be expected, since the older analyses relied upon determination of the weights of ether- and petrol-insoluble bromides, a procedure which is now known to be uncertain and of no great accuracy.

TABLE III.

Composition of the mixed acids of Œnothera biennis *oil, excluding unsaponifiable matter* (% *by wt.*).

	Present work.	Heiduschka and Luft.	Eibner and Schild.
Hexoic	—	0·8	—
Palmitic	8·8	5·5	5·7
Stearic	1·3		
C_{22}—C_{24}	1·0		
Oleic	7·0	27·2	26·4
Linoleic	71·7	64·1	58·2
Octadeca-6 : 9 : 12-trienoic	10·2	2·4	9·7

Though hexoic acid has been sought in the oil, no trace has been found, and that detected by Heiduschka *et al.* was probably an oxidative fission product of either the linoleic or the octadeca-6 : 9 : 12-trienoic acid in the oil.

The bright yellow unsaponifiable matter in the oil has been shown by Klapholz and Zelbner (*Monatsh.*, 1926, **47**, 174) to be a mixture of phytosterols.

EXPERIMENTAL.

Plants growing on the sand-dunes at Birkdale, Lancashire, were collected in late October and air-dried at room temperature for a fortnight. The seeds (average weight 0·48 mg.) were separated by hand from the pods and dried to constant weight at 100°. Extraction of the dried seeds with light petroleum (b. p. 40—60°) yielded 11·1% (expressed on dried seeds) of a clear yellow oil (see Table I).

The mixed fatty acids (96·85 g.; I.V., 163·3) prepared by hydrolysis of the oil with alcoholic potash were crystallised twice at −65° from a 9% solution in acetone, and the insoluble acids recrystallised first at −60°, and then at −30°, from a 9% solution in ether with the following results.

Fraction.	Conditions.	G.	%.	I.V.
A	Insoluble in ether at −30°	8·07	8·3	5·9
B	Soluble in ether at −30°	3·75	3·9	145·3
C	Soluble in ether at −60°	58·61	60·5	170·9
D	Soluble in acetone at −65°	6·05	6·3	175·7
E	Soluble in acetone at −65°	20·37	21·0	201·5 *

* Iodine value, after removal of unsaponifiable matter, 207·0.

The mixed acids of the oil, and the above five fractions, were examined spectroscopically after alkali isomerisation at 170° for 15 minutes and at 180° for 60 minutes by the method of Hilditch, Morton, and Riley (*loc. cit.*), with the results shown below.

	$E^{1\%}_{1\text{ cm.}}$ at 268 mμ.	$E^{1\%}_{1\text{ cm.}}$ at 234 mμ.	6 : 9 : 12-Linolenic, %.	Linoleic, %.	Oleic, %.	Saturated, %.	Unsaponifiable, %.
Mixed acids	56·7	690	10·9	69·0	9·4	10·7	
A	—	28	—	3·1	0·3	96·6	
B	0·8	679	—	74·9	7·0	15·8	2·3
C	4·9	803	0·9	88·0	6·0	2·7	2·4
D	62·3	741	11·9	73·8	6·4	5·5	2·4
E *	229	643	43·9	41·7	12·5	1·9	—

* After removal of 8·1% unsaponifiable matter (of iodine value 135·6).

Fractionation of the hydrogenated methyl esters prepared from the mixed acids showed the oil to contain 8·7% of palmitic acid and 3·6% of unsaponifiable matter, and less than 0·03% of acid lower than palmitic.

The above data, combined with the results obtained by fractional distillation of the methyl esters of the acids A (for details see below) showed the composition of the mixed acids of the oil to be as follows:

	A (8·3%).	B (3·9%).	C (60·5%).	D (6·3%).	E (21·0%).	Total.	%, Excluding unsaponifiable.
Unsaponifiable	0·12	0·09	1·45	0·15	1·70	3·51	—
Palmitic	5·53	0·62	1·63	0·35	0·37	8·50	8·8
Stearic	1·32	—	—	—	—	1·32	1·3
As behenic	1·00	—	—	—	—	1·00	1·0
Oleic	0·02	0·27	3·63	0·40	2·41	6·73	7·0
Linoleic	0·31	2·92	53·25	4·65	8·05	69·18	71·7
6 : 9 : 12-Linolenic	—	—	0·54	0·75	8·47	9·76	10·2

Examination of Fraction A.—The methyl esters of fraction A on distillation yielded fractions having the following characteristics :

Fraction.	g.	Sap. equiv.	I.V.	Fraction.	g.	Sap. equiv.	I.V.
A1	1·85	269·7	0·4	A3	1·92	287·3	16·3
A2	2·03	266·4	2·1	A4	0·95	389·7	10·0

Fraction A1 was converted into its *p*-bromophenacyl ester, which after recrystallisation had m. p. 83—84° (no depression with the *p*-bromophenacyl derivative of palmitic acid, m. p. 83·5—84°).

Fraction A3 was hydrolysed, and the resultant acid recrystallised three times from ethyl acetate, giving an acid, m. p. 65—66° [mixed m. p. with stearic acid (m. p. 69°), 65—68°]; the compound was therefore a somewhat impure stearic acid.

Fraction A4. The acids (sap. equiv., 339·4; m. p. 67°) obtained by saponification of A4 and removal of unsaponifiable matter could not be further resolved by crystallisation and were therefore probably a ternary mixture of arachidic, behenic, and lignoceric acids.

Examination of Fraction E.—The unsaponifiable matter was removed from 19·5 g. of E by the Society of Public Analysts method (*Analyst*, 1933, **58**, 203), yielding 17·1 g. of acids, I.V. 207·0, $E^{1\%}_{1\,\text{cm.}}$ at 234 mμ., 6·1; at 268 mμ., 0·8 (on unisomerised acids, indicating absence of conjugated di- or tri-ene acids), and 1·58 g. (8·1%) of unsaponifiable matter, I.V. 135·6.

Ozonolysis of E (after Removal of Unsaponifiable Matter).—1·025 G. of E were ozonised in 25 ml. of chloroform at −70° for 1 hour, and the chloroform then removed by passing dry air through the solution. The ozonide was hydrolysed by boiling under reflux with 50 ml. of water for 2 hours, and the resultant aldehydes oxidised by treatment with 3 ml. of 30% hydrogen peroxide. The liquid was evaporated to dryness, and the crude dibasic acids (0·474 g.) recrystallised six times from ether, yielding 0·05 g. of adipic acid, m. p. 147° [mixed m. p. with adipic acid (m. p. 151°), 148—150·5°].

*Preparation of Methyl Octadeca-*6 : 9 : 12*-trienoate.*—The acids E (15·07 g.) (after removal of unsaponifiable matter) were brominated in solution in 150 ml. of ether at below −10° with 6·3 ml. of bromine. The insoluble bromides (7·36 g.) were filtered off and washed with ether; they were then crushed and boiled under reflux three times with ether (200 ml.) to dissolve tetrabromides. There remained 5·85 g. of ether-insoluble bromides, m. p. 196° [Found : Br, 62·4 (determined by Stepanow's method (*Ber.*, 1906, **39**, 4056). Calc. for hexabromostearic acid : Br, 63·3%].

5·5 G. of the hexabromostearic acid were debrominated with 6 g. of zinc dust in 30 ml. of pyridine according to the method of Kaufmann and Mestern (*Ber.*, 1936, **69**, 2684). The debrominated acid (1·86 g.) was methylated overnight at 0° with 20 ml. of methyl alcohol containing 0·5% of hydrogen chloride, which yielded 1·81 g. of neutral ester; this was fractionated at *ca.* 0·1 mm. through a micro-distillation apparatus, yielding 0·92 g. of methyl octadeca-6 : 9 : 12-trienoate (Found : sap. equiv., 292·9; I.V., 261·5. Calc. : sap. equiv., 292·0; I.V., 260·9). The pure ester was subjected to alkali isomerisation at 170° for 15 minutes and at 180° for 60 minutes according to the method of Hilditch, Morton, and Riley (*loc. cit.*), and the spectrographic values of the derived acids were determined: $E^{1\%}_{1\,\text{cm.}}$ at 268 mμ. (170°/15 mins.), 522; $E^{1\%}_{1\,\text{cm.}}$ at 234 mμ. (180°/60 mins.), 603.

Identification of Oleic and Linoleic Acids.—After removal of unsaponifiable matter, the mixed acids (12·75 g.) from the oil were recrystallised from a 9% solution in acetone at −65°, and the resultant deposited crystalline acids (S1) (10·44 g.; I.V., 154·9) were employed for the identification of oleic and linoleic acids.

(i) *Acetone–permanganate oxidation of* S_1. The methyl ester of S_1 (2·59 g.) was oxidised in solution in acetone with 15 g. of potassium permanganate. There was recovered 0·68 g. of crude dibasic acids, which, on recrystallisation from water yielded 0·27 g. of azelaic acid, m. p. 103—104° (undepressed on admixture with authentic azelaic acid, m. p. 103—104°).

(ii) *Bromination.* S_1 (2·37 g.) was brominated in 5% solution in light petroleum (b. p. 40—60°) at −10° with 0·79 ml. of bromine. The resultant crystalline bromides (2·029 g.) were recrystallised from ether, yielding 0·85 g. of tetrabromostearic acid, m. p. 114—115° (Found: Br, 52·8. Calc. for tetrabromostearic acid : Br, 53·3%) (undepressed on admixture with 9 : 10 : 12 : 13-tetrabromostearic acid, m. p. 114—115°).

(iii) *Lapworth–Mottram oxidation.* S_1 (6·10 g.) was oxidised with 600 ml. of 1% aqueous potassium permanganate solution according to Lapworth and Mottram's procedure (*J.*, 1925, **127**, 1628). There were recovered 4·12 g. of hydroxy-acids and 0·39 g. of saturated acids. On recrystallisation of the hydroxy-acids from water and ethyl acetate, 0·12 g. of dihydroxystearic acid, m. p. 129—130°, was obtained (m. p. undepressed on admixture with 9 : 10-dihydroxystearic acid, m. p. 131°). In addition, the two 9 : 10 : 12 : 13-tetrahydroxystearic acids, m. p. 155° (0·10 g.) and m. p. 169—170° (1·02 g.), formed from linoleic acid were also recovered from the hydroxy-acids.

The author wishes to thank Professor T. P. Hilditch, F.R.S., for his valuable suggestions and guidance throughout this work.

DEPARTMENT OF INDUSTRIAL CHEMISTRY,
THE UNIVERSITY, LIVERPOOL.

[*Received, May 2nd,* 1949.]